



National Focal point of Slovak Republic for scientific and technical matters for EFSA –
Ministry of Agriculture and Rural Development of the Slovak Republic, Bratislava

Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava

National Focal point of Czech Republic for scientific and technical matters for EFSA –
Ministry of Agriculture of the Czech Republic, Prague

National Focal point of Austria for scientific and technical matters for EFSA –
the Austrian Agency for Health and Food Safety (AGES), Vienna

Conference proceedings

from

scientific conference with international participation on

ENDOCRINE DISRUPTORS

BRATISLAVA 16th – 17th of October 2012

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Doc. Ing. Lubos Babicka, CSc., Czech University of Life Sciences Prague, Czech Republic
prof. MUDr. Jozef Bires, DrSc., Chief Veterinary Officer of the Slovak Republic
Dipl. Ing. Zuzana Birosova, CSc., Director of Food Safety and Nutrition Department,
Ministry of Agriculture and Rural Development, Slovak Republic
Dipl. Ing. Julius Brtko, DrSc., Guarantor of Biochemistry, Institute of Experimental Endocrinology
Slovak Academy of Sciences, Slovak Republic
Dipl. Ing. Dr. Roland Grossgut, Austrian Agency for Health and Food Safety, Austria
prof. MUDr. Juraj Payer, PhD., Chief Endocrinologist of the Slovak Republic

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Program of lectures and posters

PROGRAM
Lectures
16th of October, 2012

- 09: 00 – 09: 20 h** **OPENING OF THE CONFERENCE**
- Dipl. Ing. Zuzana Birosova, CSc., *Head of National Focal point for EFSA in the Slovak Republic, Ministry of Agriculture and Rural Development of the Slovak Republic*
- Dipl. Ing. Jitka Götzova, *Head of National Focal point for EFSA in the Czech Republic, Ministry of Agriculture of the Czech Republic*
- Dipl. Ing. Dr. Roland Grossgut, *Alternate of National Focal point for EFSA in Austria, Austrian Agency for Health and Food Safety*

Chairmans: Dipl.Ing.Dr. Roland Grossgut, Dipl. Ing. Zuzana Birosova,CSc.
- 09: 20 – 09. 45 h** **Endocrine disruptors and food safety – a risk assessor’s view**
Pratt I.
Food Safety Authority of Ireland
- 09: 45 – 10: 10 h** **Endocrine disrupters in mothers and their newborn**
The Um-MuKi Bratislava-Vienna study I: Organic and organometallic compounds
Uhl M.¹, Gundacker C.², Gencik M.³, Graf- Rohrmeister C.⁴, Hartmann C.¹, Holomann K.⁵, Offenthaler I.¹, Köhler-Vallant B.¹, Kroismayr R.¹, Raffesberg W.¹, Scharf S.¹, Vargova P.⁵, Vlckova Z.³, Weiß S.¹
¹*Environment Agency Austria, Vienna*
²*Institute of Medical Genetics, Medical University of Vienna*
³*MedGene, Bratislava*
⁴*Semmelweis Frauenklinik, Vienna*
⁵*University Hospital of Ruzinov, Bratislava*
- 10: 10 – 10: 35 h** **Endocrine Disrupters in Mothers and Newborns:**
The Um-MuKi Bratislava-Vienna study II: Heavy metals
Gundacker C.¹, Graf- Rohrmeister K.², Teufl I.¹, Reischer T.¹, Plichta V.¹, Scheinast M.¹, Damjanovic L.¹, Holomann C.³, Vargova P.³, Vlckova Z.⁴, Gencik M.⁴, Uhl M.⁵
¹*Institute of Medical Genetics, Medical University of Vienna*
²*Semmelweis Frauenklinik, Vienna*
³*University Hospital of Ruzinov, Bratislava*
⁴*MedGene, Bratislava*
⁵*Environment Agency Austria, Vienna*
- 10: 35 – 11. 00 h** **Endocrine Disrupters in Mothers and Newborns:**
The Um-MuKi Bratislava-Vienna study III: Genetic aspects
Gencik M.¹, Uhl M.², Graf- Rohrmeister K.³, Behalova L.¹, Holoman K.⁴, Kroismayer R.², Vargova P.⁴, Vlckova, Z.¹, Gundacker C.⁵
¹*MedGene, Bratislava*
²*Environment Agency Austria, Vienna*
³*Semmelweis Frauenklinik, Vienna*
⁴*University Hospital of Ruzinov, Bratislava*
⁵*Institute of Medical Genetics, Medical University of Vienna*
- 11: 00 – 11: 25 h** **C O F F E E B R E A K** coupled with poster tour
- 11: 25 – 11: 45 h** **Possibilities of scientific cooperation with EFSA**
Moon J.
European Food Safety Authority, Advisory Forum & Scientific Cooperation Unit, Parma

- 11: 45 – 12: 00 h** ***In vivo* effect of selected metals on the activity of selenoenzyme iodothyronine 5'-deiodinase in rat liver**
Brtko J.¹, Macejova D.¹, Kotyzova D.², Eybl V.²
¹*Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava*
²*Department of Pharmacology and Toxicology, Charles University in Prague, Faculty of Medicine in Pilsen*
- 12: 00 – 13: 25 h** **L U N C H**
Chairmans: prof.RNDr. Zdenek Dvorak, PhD., Dipl.Ing.Julius Brtko, DrSc.
- 13: 30 – 13: 50 h** **Occurrence Data of Bisphenol A and Phthalates in food and related products in Austria**
Grossgut R.
Austrian Agency for Health and Food Safety, Data, Statistics and Risk Assessment, Vienna
- 13: 50 – 14: 10 h** **Bisphenol A modifies proliferation of human breast carcinoma cells**
Fickova M., Havranek T., Macejova D., Brtko J., Mlynarcikova A.,
Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava
- 14: 10 – 14: 30 h** **Bisphenol A in food contact materials (FCM) – determination and performing the official controls in the Slovak Republic**
Sycova M., Rosipal R.
Regional Public Health Authority in Poprad, NRL for food contact materials, Poprad
- 14: 30 – 14: 50 h** **Occurrence of endocrine-disrupting pesticide residues in samples intended for official control**
Matusova M.
State Veterinary and Food Administration of the Slovak Republic, Bratislava
- 14: 50 – 15: 20 h** **C O F F E E B R E A K coupled with poster tour**
- 15: 20 – 15: 45 h** **Construction and characterization of stably gene reporter cell lines for detection of endocrine disruptors**
Dvorak Z.¹, Pavek P.², Novotna A.¹
¹*Department of Cell Biology and Genetics, Faculty of Science, Palacky University, Olomouc*
²*Department of Pharmacology and Toxicology, Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Hradec Kralove*
- 15: 45 – 16: 05 h** **Current situation of research of endocrine disruptors occurring in articles for common use and health risks of consumers evaluation in the Czech Republic**
Sosnovcova J., Rucki M., Vrbik K.
National Institute of Public Health, Prague
- 16: 05 – 16: 20 h** ***In vitro* effects of selected isoflavones on target genes expression in human MCF-7 cell line**
Bialesova L., Brtko J., Macejova D.
Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava
- 16: 20 – 16: 40 h** **Folic acid and its impact on newborns**
Zikavska T., Brucknerova I.
^{1st} *Department of Paediatrics, Faculty of Medicine, Comenius University in Bratislava*
- 16: 40 – 17: 00 h** **Lapatinib inhibits meiotic maturation of oocyte – cumulus complexes cultured *in vitro* in gonadotropins – supplemented medium**
Nagyova E.
Institute of Animal Physiology and Genetics Academy of Sciences of the Czech Republic, Libechov
- 19: 30 – 23: 00 h** **S O C I A L E V E N T**

P R O G R A M
Lectures
17th of October, 2012

Chairmans: RNDr. Miroslav Machala, CSc., Mgr. Jozef Ukropec, PhD.

- 09: 00 – 09: 25 h** **Heavy metals as endocrine disruptors in the model system**
Lukac N., Forgacs Zs.¹, Knazicka Z., Lukacova J.
Department of Animal Physiology, Faculty of Biotechnology and Food Sciences Slovak University of Agriculture, Nitra
¹*National Institute of Chemical Safety, "Jozsef Fodor" National Center for Public Health, Budapest*
- 09: 25 – 09: 50 h** **Disorders of thyroid and male gonads function in the population of East Slovakia**
Langer P.¹, Tajtakova M.², Kocan A.³, Drobna B.³, Huckova M.¹, Trnovec T.³, Klimes I.¹
¹*Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava*
²*The First Clinic of Internal Medicine, P.J. Safarik University, Kosice*
³*Department of Toxic Organic Pollutants, Slovak Medical University, Bratislava*
- 09: 50 – 10: 15 h** **Could environmental pollution with persistent organic pollutants affect our metabolic health?**
Ukropec J.¹, Radikova Z.², Huckova M.¹, Koska J.², Kocan A.³, Sebokova E.¹, Drobna B.³, Trnovec T.³, Susienkova K.⁴, Gasperikova D.¹, Langer P.¹, Klimes I.¹
¹*Diabetes Laboratory & ²Laboratory of Human Endocrinology, Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava*
³*Department of Toxic Organic Pollutants, Slovak Medical University, Bratislava*
⁴*Department of Statistics, Faculty of Economic Informatics, Economics University, Bratislava*
- 10: 15 – 10: 45 h** **Endocrine – disrupting activities of PCBs and other food contaminants**
Machala M., Vondracek J.
Veterinary Research Institute, Brno
- 10: 45 – 11: 10 h** **C O F F E E B R E A K** coupled with poster tour
- 11: 10 – 11: 35 h** **Relation of cord to maternal serum concentration ratios of individual PCB congeners to their physico-chemical parameters**
Lancz K., Palkovicova L., Patayova H., Wimmerova S., Trnovec T.
Slovak Medical University, Bratislava
- 11: 35 – 12: 00 h** **Relationship between anthropometric, socioeconomic and health characteristics of mother and cord/maternal serum organochlorine compounds concentration ratio**
Patayova H., Wimmerova S., Lancz K., Palkovicova L., Drobna B., Hertz-Picciotto I., Jusko T. A., Trnovec T.
Slovak Medical University, Bratislava
- 12: 00 – 12: 30 h** **Closure of the conference**

P R O G R A M
Posters

- 1. The endocrine disruptive effects of copper on the steroidogenesis in the model system NCI – H295R**
Knazicka Z., Lukacova J., Lukac N.
Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Nitra
- 2. Cu²⁺ with glucose impair insulin binding to its receptor in rat liver plasma membrane**
Zorad S.¹, Krskova K.¹, Gajdosechova L.¹, Dovinova I.²
¹*Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava*
²*Institute of Normal and Pathological Physiology Slovak Academy of Sciences, Bratislava*
- 3. Effect of copper complex on DNA cleavage and toxicity *in vitro***
Dovinova I.¹, Gajdosechova L.², Zorad S.²
¹*Institute of Normal and Pathological Physiology Slovak Academy of Sciences, Bratislava*
²*Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava*
- 4. Prenatal and postnatal PCB exposure of non – breastfed infants from Michalovce district and their neurodevelopment**
Fabisikova A., Drobna B., Conka K., Chovancova J., Dömötörova M., Kocan A.
Department of Toxic Organic Pollutants, Faculty of Medicine, Slovak Medical University, Bratislava
- 5. Persistent organic pollutants in food samples from potential sources of contamination in Slovakia**
Conka K., Fabisikova A., Chovancova J., Dömötörova M., Stachova Z., Drobna B., Kocan A.
Slovak Medical University, Bratislava
- 6. Effects of selected endocrine disruptors on porcine oocyte-cumulus complex expansion and oocyte maturation**
Mlynarcikova A., Fickova M., Scsukova S.
Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava
- 7. Effects of selected endocrine disruptors on steroidogenesis in ovarian cells**
Scsukova S., Fickova M., Mlynarcikova A.
Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava
- 8. Impact of Polymeric Nanoparticle PEG-*b*-PLA on Rat Hypothalamic-Pituitary-Gonadal Functions**
Rollerova E.¹, Scsukova S.², Jurcovicova J.², Mlynarcikova A.², Kiss A.², Wsolova L.¹, Kovriznych J.¹, Zeljenkova D.¹, Szabova E.¹, Vavra I.³, Ciampor F.⁴, Sadlonova I.⁵
¹*Department of Toxicology, Slovak Medical University, Bratislava*
²*Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava*
³*Institute of Electrical Engineering Slovak Academy of Sciences, Bratislava*
⁴*Institute of Virology Academy of Sciences, Bratislava*
⁵*Hamel n rds a.s., Modra*
- 9. Effects of vinclozolin on selected nuclear receptors and their coregulators expression in human MCF-7 cells**
Macejova D., Fickova M., Brtko J.
Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava
- 10. The effect of 4-nonylphenol, an endocrine disruptor, with the protective effect of 17β-estradiol on spermatozoa *in vitro***
Lukacova J., Knazicka Z., Tvrda E., Lukac N.
Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Nitra
- 11. Pan-European campaigns on EDCs in ground-, surface- and waste-waters**
Blaha L., Jarosova B., Benisek M., Hilscherova K., Giesy J., Loos R., Gawlik B.
RECETOX, Masaryk University, Brno

12. Endocrine disruptive potential of cyanobacterial metabolites

Hilscherova K., Novakova K., Jonas A., Sychrova E., Fetter E., Scholz S.
RECETOX, Masaryk University, Brno

Abstracts

ENDOCRINE DISRUPTORS AND FOOD SAFETY – A RISK ASSESSOR’S VIEW

Pratt I.

Food Safety Authority of Ireland and Chair of EFSA’s Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids

The potential effects of endocrine-disrupting chemicals on human health and on ecosystems have been hotly debated over several decades. Studies reporting effects on the endocrine system at doses below established health-based guidance values have been particularly controversial, since such studies challenge the current risk assessment paradigm and the safety of certain chemicals occurring widely in consumer products, food and/or the environment.

In addition to low dose effects, endocrine disruptors have also been reported to show non-monotonic dose responses (NMDR), further challenging the classical risk assessment paradigm which is based primarily on the concept of a threshold dose below which no adverse effect effects on health and/or the environment are identified.

The scientific evidence for low dose effects and NMDR has largely derived from academic studies carried out by experimental scientists, and acceptance of their use in risk assessment has been slow, due to the uncertainties regarding hazard identification and hazard as well as risk characterization for such effects.

Progress is being made, however, spear-headed by several international meetings on the topic and the need, both within the EU and internationally, to agree on approaches for the identification and risk assessment of endocrine disruptors. The presentation will provide an overview of these issues and the problems arising in the risk assessment of food chemicals.

ENDOCRINE DISRUPTERS IN MOTHERS AND THEIR NEWBORN

The Um-MuKi Bratislava-Vienna study I: Organic and organometallic compounds

Uhl M.¹, Gundacker C.², Gencik M.³, Graf-Rohrmeister C.⁴, Hartmann C.¹, Holomann K.⁵, Offenthaler I.¹, Köhler-Vallant B.¹, Kroismayr R.¹, Raffesberg W.¹, Scharf S.¹, Vargova P.⁵, Vlckova Z.³, Weiß S.¹

¹Environment Agency Austria, Vienna, ²Institute of Medical Genetics, Medical University of Vienna, ³MedGene, Bratislava, ⁴Semmelweis Frauenklinik, Vienna, ⁵University Hospital of Ruzinov, Bratislava

Introduction: Nowadays, it is scientifically accepted that exposures within the fetal period can lead to adverse effects on lifetime health. Regulatory frameworks should guarantee that exposure to chemicals in our environment does not harm the developing child; however their effectiveness has to be controlled. Human biomonitoring, the detection of chemicals and their metabolites in body fluids, allows determining the total body burden via all pathways (e.g. food, cosmetics, consumer products, life style, indoor and environmental pollution) and the risks for the individual or population.

Aim of the study: Within this transnational cooperation project a human biomonitoring study was performed in two cities which are at 50 km airline distance in two countries: Bratislava and Vienna. The concentrations of methyl mercury, perfluorinated substances and bisphenol A were analysed in 40 mother-child pairs, 20 in each city, whereas heavy metals were analysed in total in 200 mother-child pairs.

Methodology: Chemical analyses were performed in the accredited human biomonitoring laboratory of the Environment Agency Austria. Methyl mercury was measured in 1 ml of blood of the newborn with HPLC-ICPMS. Perfluorinated compounds and Bisphenol A were measured in blood serum and cord blood with HPLCMS-MS in negative mode. Environmental and lifestyle parameters were surveyed with questionnaires (set in relation to questionnaires in order) to identify potential risk factors. Data were also examined for differences between Austrian and Slovakian mother-child pairs.

Results: The median concentration of Me-Hg in blood of Slovakian (Austrian) newborns was 0.35 (1.3) µg/l; and in their mothers 0.35 (0.73) µg/l. In Slovakian (Austrian) mother-child pairs the median PFOS concentration was 1.3 (1.8) ng/ml in maternal blood and 0.2 (0.6) ng/ml in cord blood. Median PFOA levels in cord blood from Slovakia (Austria) were in maternal blood 4.9 (1.7) ng/ml, and 2.7 (1.3) ng/ml. In Slovakia (Austria) 8 (13) maternal samples and 10 (18) cord blood samples had no detectable concentrations of free BPA, the concentration ranges were 0.3-3.3 (0.4-1.7) ng/ml in maternal blood and 0.3 - 3.8 (0.3 - 1.9) ng/ml in cord blood.

Conclusion: Babies in the womb are exposed to various endocrine disrupters demonstrating the need for prevention; this study shows that differences in life style and environmental influences can lead to considerably different exposure levels and suggests recommendations of prevention measures to mothers.

The study has been funded by the European Cross-Border Programme Slovakia-Austria 2007-2013.

ENDOCRINE DISRUPTERS IN MOTHERS AND NEWBORNS:

The Um-MuKi Bratislava-Vienna study II: Heavy metals

Gundacker C.¹, Graf-Rohrmeister K.², Teufel I.¹, Reischer T.¹, Plichta V.¹, Scheinast M.¹, Damjanovic L.¹, Holomann C.³, Vargova P.³, Vlckova Z.⁴, Gencik M.⁴, Uhl M.⁵

¹Institute of Medical Genetics, Medical University of Vienna, ²Semmelweis Frauenklinik, Vienna, ³University Hospital of Ruzinov, Bratislava, ⁴MedGene, Bratislava, ⁵Environment Agency Austria, Vienna

Introduction: Prenatal exposure to the heavy metals mercury (Hg) and lead (Pb) can adversely affect fetal health particularly brain development. In addition, birth weight and birth length might be reduced due to fetal Pb exposure. The situation for perinatal Hg and Pb exposures in the Vienna-Bratislava region is underexplored.

Aim of the study: To determine the population-specific sources of perinatal mercury and lead exposures.

Methodology: 100 healthy mother-child pairs in Bratislava and in Vienna, respectively, participated in the Um-MuKi study conducted between April 2010 and December 2011. Total Hg and Pb concentrations were measured in maternal blood erythrocytes (MatBl_E), maternal hair (MatHair), and cord blood erythrocytes (CordBl_E) by CV-AFS and GF-AAS. Maternal anthropometry, nutrition, smoking habits, and dental status were surveyed by questionnaire. The medical records provided data on pregnancy outcome and on newborn anthropometry. Kruskal-Wallis test and Spearman correlation were applied in statistical analyses, i.e., group comparisons and correlation analyses.

Results: We found significant correlations between MatBl_E-Hg and CordBl_E-Hg and between MatBl_E-Pb and CordBl_E-Pb ($r > 0.5$, $P < 0.001$, respectively). During pregnancy the Viennese women were consuming more fish as compared to women in Bratislava ($P < 0.05$). This may well explain the significantly higher Hg loads of children born in Vienna ($P < 0.001$). Further factors associated with perinatal Hg exposure were maternal age, maternal education level, and number of dental amalgam fillings ($P < 0.05$, respectively). In Vienna MatBl_E-Pb and CordBl_E-Pb levels are higher than in Bratislava ($P < 0.05$, respectively). This might be explained by the higher rate of tap water consumption in Vienna and by the fact that the Viennese participants are living more often in old buildings, where leaded water pipes are still in use. Maternal age and smoking habits are further factors influencing perinatal Pb concentrations ($P < 0.05$, respectively). Hg and Pb concentrations were not associated with birth weight, birth length, and head circumference ($P < 0.05$, respectively).

Conclusion: Our data are the basis for developing the appropriate population-specific preventive measures, which are required to protect the fetus from unhealthy Hg and Pb exposures.

The study has been funded by the European Cross-Border Programme Slovakia-Austria 2007-2013.

ENDOCRINE DISRUPTERS IN MOTHERS AND NEWBORNS:

The Um-MuKi Bratislava-Vienna study III: Genetic aspects

Gencik M.¹, Uhl M.², Graf-Rohrmeister K.³, Behalova L.¹, Holoman K.⁴, Kroismayer R.², Vargova P.⁴, Vlckova, Z.¹, Gundacker C.⁵

¹MedGene, Bratislava, ²Environment Agency Austria, Vienna, ³Semmelweis Frauenklinik, Vienna, ⁴University Hospital of Ruzinov, Bratislava, ⁵Institute of Medical Genetics, Medical University of Vienna

Introduction: Both maternal and fetal genetic make-up influence the physiology and functioning of the placental barrier. Little is known about the epistatic effects of both genomes on nutrient and vitamin supply for the fetus and on prenatal exposure to xenobiotics.

Aim of the study: To determine the genetic variants influencing lead, mercury, bisphenol A (BPA) and perfluorated tenside levels in pregnant women and fetuses. To identify potential risk for pregnancies in dependence of the genotype(s). For this purposes, 100 mother-child pairs from Vienna and 100 mother-child pairs from Bratislava were investigated.

Methodology: We have isolated DNA from blood of 200 mother-newborn pairs. Each individual was genotyped for 26 SNPs in candidate genes with possible involvement in the metabolism of heavy metals (lead, total mercury, methyl mercury), BPA and perfluorated tensides (i.e., *VDR*, *ALAD*, *HFE*, *MT1-4*, *GCLC*, *GCLM*, *GSTT1*, *GSTM1*, *GSTP1*, *CYP7A1*, *UGT2B*, *ABCB1*, *ABCC1*, *ABCC2*, *ABCG1*, *ABCG2*, *ABCB11*) by means of TaqMan-based real-time PCR. Genotypes were examined in respect to pollutant levels and pregnancy outcome.

Results: Our study provides several important insights into the complex interplay between maternal-fetal genetic backgrounds and perinatal exposure levels. The genetic background of Slovak and Austrian women is similar. In our analyses on gene-environment interactions we therefore do not discriminate between the Bratislava and the Vienna group. So far we observed that just a few SNPs are associated with lead, mercury, BPA, and perfluorated tenside levels. In our ongoing statistical analyses we focus on those individuals whose exposure levels cannot be explained by the known exposure factors, for instance, on mother-child-pairs whose mercury loads cannot be explained by maternal fish and seafood consumption. We hypothesize that exposure levels in such individuals are influenced by a certain maternal-fetal genetic background, which modulates transfer of xenobiotics across the placenta and in this way may influence intrauterine development and fetal health.

Conclusion: This work serves as a pilot study in the Bratislava-Vienna region, brings this issue to general awareness and shows that it is worth to further pursue genotype-phenotype association studies with mother-child-pairs. Our findings might enable us to develop the appropriate preventive measures in order to minimize prenatal exposure to xenobiotics in genetically susceptible individuals.

The study has been funded by the European Cross-Border Programme Slovakia-Austria 2007-2013.

POSSIBILITIES OF SCIENTIFIC COOPERATION WITH EFSA

Moon J.

European Food Safety Authority, Advisory Forum & Scientific Cooperation Unit, Parma

The presentation on Scientific Cooperation will explain the general arrangements of EFSA and the possibilities for cooperation, including details of some of the tools used.

Cooperation with Networks and Partners:

The European Food Safety Authority is the keystone of European Union risk assessment regarding food and feed safety. To accomplish its mission, EFSA works closely with partners and stakeholders, and is a proactive member of important networks.

Partners include institutions with whom the Authority has a legal obligation to work with under EU rules specifically risk managers working within the European Commission, the European Parliament and the Member States, and stakeholder groups and individuals or groups who feel they can contribute to the Authority's work.

Networks and specific projects where EFSA plays an important role include the Instrument for Pre- Accession Assistance, financed by the European Union to assist candidates and potential candidate countries in their preparations for joining the European Union, and international cooperation through involvement with bodies such as the Codex Alimentarius Commission.

EFSA also organises and participates in many events annually on scientific topics within its mandate. These include workshops, conferences and roundtables. These enable EFSA to update partners and interested parties on new developments on scientific subjects within its remit as well as to gather feedback, information and different points of view on ongoing work such as the development of guidance documents or risk assessments.

Networks of scientific organisations supporting EFSA units:

EFSA's networks consist of nationally appointed EU Member State organisations with expertise in the fields covered by the network. Representatives of the commission and of other organisations, including those from outside the EU with specific expertise may also be invited to participate in the work of EFSA networks.

Networks are chaired by EFSA and supported by the relevant EFSA unit. Their aim is to facilitate scientific cooperation in the fields of EFSA's mission by:

- Coordinating activities
- Exchanging information
- Developing and implementing joint projects
- Exchanging expertise and best practices.

Article 36 Cooperation:

One of EFSA's priorities is enhanced cooperation and networking in Europe.

In line with Article 36 of EFSA's Founding Regulation and its implementing rules, EFSA's Management Board approved a list of organisations capable of assisting the Authority in its tasks in December 2006. Networking with these competent organisations enables EFSA to use a wider spectrum of scientific excellence in Europe. This helps EFSA respond more effectively and flexibly to its growing workload.

EFSA may entrust these organisations with the following tasks:

- Data collection
- Preparatory work for scientific opinions
- Other scientific and technical assistance.

EFSA may award grants to these organisations through calls for proposals to support them in carrying out the requested tasks. EFSA publishes indicative information on the activity areas, budget and timing for calls planned under EFSA's Annual Work Programme for Grants. The organisations included in the list are designated by Member States, with the support of Focal Points. Since organisations evolve and needs may change in response to scientific and policy developments, the list is regularly reviewed.

Expert Database:

EFSA has established a database of external scientific experts able to assist its Scientific Committee, Scientific Panels, EFSA networks and respective working groups.

Scientists with relevant expertise are invited to apply. EFSA welcomes applications from experts in all fields of its remit, such as food and feed safety, nutrition, toxicology, chemistry, animal health and welfare, plant protection and plant health.

All candidates who submit a complete application and meet the eligibility criteria will be included in the database. EFSA informs each applicant whether they have been included and, if not, the criteria that were not met.

A report has been produced summarising the activities carried out during 2011 to implement the expert database project. Included in the report is an assessment of the information available in the database at the end of 2011 compared with 2010.

IN VIVO EFFECT OF SELECTED METALS ON THE ACTIVITY OF SELENOENZYME IODOTHYRONINE 5'-DEIODINASE IN RAT LIVER

Brtko J.¹, Macejova D.¹, Kotyzova D.², Eybl V.²

¹*Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava,* ²*Department of Pharmacology and Toxicology, Charles University in Prague, Faculty of Medicine in Pilsen*

Introduction: In general, iodothyronine deiodinases are envisaged as “guardian to the gate” of thyroid hormone action mediated by 3,5,3'-triiodothyronine (T₃) – activated nuclear receptors in target tissues. Type I iodothyronine deiodinase (5'-DI), one of three selenium containing isoenzymes, generates the thyromimetically active hormone T₃ by reductive monodeiodination at the 5'-position of the phenolic ring of L-thyroxine (T₄).

Aim of the study: The present study was undertaken in order to compare the in vivo effects of selected environmental metal pollutants with selected essential trace elements on the activity of 5'-DI in the liver of rat.

Methodology: (1) Male CD rats (Charles River, Germany) were treated i.p. either with CdCl₂, Na₂HASO₄ or Pb(CH₃COO)₂ (0.01 mmol/kg) five times a week for six weeks. In the next groups of rats 0.0025 mmol/kg or 0.0050 mmol/kg HgCl₂ were administered i.p. five times a week during a period of six weeks. (2) Male CD rats were treated either with Na₂SeO₃ (3 mg Se/L), Na₂TeO₃ (3 mg Te/L) or Na₂SeO₃ (3 mg Se/L) + Na₂TeO₃ (3 mg Te/L) in tap water during a period of 6 weeks. (3) Male CD rats were treated either with ZnSO₄ (58 mg Zn/L), ZnSO₄ (58 mg Zn/L) + Na₂SeO₃ (3 mg Se/L) or with ZnSO₄ (58 mg Zn/L) + Na₂TeO₃ (3 mg Te/L) in tap water during a period of 6 weeks.

Results: All the toxic environmental contaminants (cadmium, arsenic^{III}, lead or mercury) in vivo caused a significant inhibition of the 5'-DI activity in liver. Selenium, and surprisingly tellurium, or a combination of selenium and tellurium in vivo significantly enhanced 5'-DI activity in rat liver. On the other hand, zinc alone was incapable to affect the activity of 5'-DI in liver, however, a combination of zinc and selenium or a combination of zinc and tellurium caused a significant increase of 5'-DI activity in the liver of rat.

Conclusions: Inhibition of type I 5'-DI by cadmium, arsenic^{III}, lead or mercury, and contrary, stimulation of 5'-DI by tellurium might be caused either by direct interaction of metal with selenium and/or by the effect of those elements on the expression of 5'-DI.

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OCURRENCE DATA OF BISPHENOL A AND PHTHALATES IN FOOD AND RELATED PRODUCTS IN AUSTRIA

Grossgut R.

Austrian Agency for Health and Food Safety, Data, Statistics and Risk Assessment, Vienna

Introduction: Phthalates may occur in food because of the use of these substances in different food contact materials or contamination via the environment. Bisphenol A is a substance, which may occur in food because of migration out of food contact materials, for which polycarbonate is used, e.g. baby bottles.

Aim of the study: To determine the occurrence of substances in food or migrates of food contact materials.

Methodology: In Austria on the one hand food is sampled by food inspectors and then analysed for different substances e.g. Phthalates. This can be done by special actions initiated by the Federal Ministry of Health. In addition food contact materials are tested and the migration of substances e.g. Bisphenol A are investigated.

Results: Phthalates were found in the past more often than now. These may be because of more strict regulations in the European Union. In addition it seems, that the food business operators carefully choose the materials, they use for containers, flexible tubes and packaging materials.

Migration of Bisphenol A occurs from baby bottles out of polycarbonate in very small amounts. In the meantime this material is not more allowed to be used in the European Union for baby bottles.

The exposition both Phthalates and Bisphenol A are in the most cases below the TDIs (tolerable daily intakes) established by scientific institutions.

Conclusion: Substances like Phthalates and Bisphenol A may occur in food because of different reasons, but the exposition will not be above the TDI in most of the cases. Some uses are forbidden now, but one should be aware, that substitution by other substances may pose some risk because of lack of knowledge regarding toxicity or occurrence.

BISPHENOL A MODIFIES PROLIFERATION OF HUMAN BREAST CARCINOMA CELLS

Fickova M., Havranek T., Macejova D., Brtko J., Mlynarcikova A.

Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava

Introduction: Increased incidence of hormone-related disturbances and cancers in women and men initiated in last two decades an intensive research focused on hormone active compounds interfering with natural hormones activities. Such exogenous substances become known as *endocrine disruptors* (ED). Xenoestrogens are a subgroup of ED implicated in estrogens and androgens actions. Bisphenol A (BPA) is chronically known as the ED with xenoestrogenic properties. The global production of 3,8 million tons (2006) makes BPA an ubiquitous chemical present in air, food, water, to which the humans are constantly and involuntary exposed. A weak estrogen like activity of individual BPA doses were repeatedly demonstrated both *in vivo* and *in vitro* experiments with proving cell growth stimulatory effects especially in human breast carcinoma cells. Humans are naturally exposed to a mixture of endogenous hormones and various EDs of exogenous origin.

Aim of the study: Therefore the aim of our study was to investigate the impact of estradiol (E2) + BPA mixture on proliferation and apoptosis pathway in human breast carcinoma cell line, MCF7 cells.

Methodology: Dose (1×10^{-15} – 1×10^{-6} M) and time (24, 48 and 72 h) dependent effects on cell growth and *de novo* DNA synthesis were determined for individual E2 and BPA; the mixture (MIX) consists of BPA (1×10^{-15} – 1×10^{-6} M) + E2 (1×10^{-12} M).

Results: A huge (~220 %) BPA stimulated DNA synthesis was detected already after 24h (>E2, >MIX), followed by elevated cell proliferation after the next 24h ($p < 0.05$, vs E2 and MIX). 48h cell treatment with MIX increased DNA synthesis was followed by elevated cell growth after the next 24h (72h total). E2 and BPA did not influenced expression of estrogen receptor (Er α) gene at any time and dose investigated, only 48h MIX treatment significantly decreased mRNA for Er α ; MIX induced reduced Er α protein levels do not correspond to gene expression changes. Expression of proapoptotic p53 protein and its gene were similarly reduced after treating the cells with MIX for 48h. Expression patterns of proapoptotic Bax gene were modified similarly as p53. A significant decrease by BPA and MIX was present (24 and 48h), while the Bax protein levels declined after 48 and 72 h treatment with MIX only. The amount of antiapoptotic Bcl-2 protein was enhanced by BPA after 48h; while 72h treatment by MIX and BPA induced higher Bcl-2 protein levels. Alterations of Bcl-2 gene expression were not confirmed. Gene expression of proapoptotic Bcl-2 has the tendency to decline in presence of MIX (24 and 48h), contrary to elevated Bcl-2 protein (48 and 72 h) levels.

Conclusion: The above results clearly indicate considerable impacts of BPA alone and even more stringent ones of the BPA mixture with estradiol on human breast carcinoma cell proliferation, wherein the participation/inhibition of apoptotic pathway proteins are involved.

The study was supported by grants APVV-0147-10, APVV-0120-07 and VEGA 2/0107/10 grants.

BISPHENOL A IN FOOD CONTACT MATERIALS (FCM) – DETERMINATION AND PERFORMING THE OFFICIAL CONTROLS IN THE SLOVAK REPUBLIC

Sycova M., Rosipal R.

Regional Public Health Authority in Poprad, NRL for food contact materials, Poprad

Introduction: BPA is used in polycarbonate, a high performance transparent, rigid plastic. Polycarbonate is used to make food containers, such as returnable beverage bottles, infant feeding (baby) bottles, tableware (plates and mugs) and storage containers. Residues of BPA are also present in epoxy resins used to make protective coatings and linings for food and beverage cans and vats. In January 2011, the European Commission adopted Directive 2011/8/EU, prohibiting the use of BPA for the manufacture of polycarbonate infant feeding bottles.

The aim of study: The aim of official controls are to determine amount of bisphenol A released from FCM.

Methodology: The level of Bisphenol A (also BADGE, BFDGE, NOGE, Bisphenol F, S) in aqueous food simulants was determined directly by high performance liquid chromatography (HPLC) multi-method with fluorescence detection (FLD-excitation:275nm, emission:305nm).

Results: The official controls activities in the field of bisphenol A started in Slovak Republic in 2009. These activities were focused on determination of bisphenol A from infant feeding bottles into food simulants (3% acetic acid and 50% ethanol) using the test conditions which are considered as a most severe. 36 baby bottles were determined and in 2011 were prohibited 112 PC baby bottles on the market. The specific monitoring programme started since 2012 under the auspices of Public Health Authority in SR which are extended on wide range of FCM (paper and board, plastic packaging materials covered by coatings and e.g.). The bisphenol A was found in some articles from paper and board.

Conclusion: The monitoring of bisphenol A is very useful tool how to safe human health.

OCCURRENCE OF ENDOCRINE-DISRUPTING PESTICIDE RESIDUES IN SAMPLES INTENDED FOR OFFICIAL CONTROL

Matusova M.

State Veterinary and Food Administration of the Slovak Republic, Bratislava

Introduction: The State Veterinary and Food Administration of the SR (SVFA SR) perform official controls of food within the competencies as laid down in the national food legislation. Pesticide residue control in food (besides baby food and infant formulae which are controlled by the Department of the Ministry of Health of the SR) is carried out in accordance with the requirements of harmonized EU legislation. Pesticide residues are particularly detected in fresh fruit and vegetables. Many pesticides detected can act as endocrine disruptors (ED).

Methodology: Sampling and analysis of pesticide residues is performed in compliance with the Multi-annual National Control Plan for Pesticide Residues in Food and Baby Food (hereinafter referred to as the “Programme”) which is annually updated pursuant to EU requirements. The Programme includes a list of pesticides and food groups that shall be monitored. Food sampling is done by inspectors of the District Veterinary and Food Administrations at all stages of the food chain. Sample analysis is carried out by the State Veterinary and Food Institute in Bratislava.

Results: A total of 300 different pesticide residues in fruit and vegetable samples were tested in 2011. From the above number, 40 pesticides can have endocrine disrupting properties that may cause adverse effects in humans. A total of 656 pesticide residue findings in fruit involved 175 residues that can be classified as ED (pyrimethanil, pyriproxyfen, captan, prochloraz, tebuconazole,...), mostly detected in table grape, lemons, apples, strawberries and tangerines. As far as vegetables, 186 pesticide residue findings involved 78 residues that can have endocrine disrupting properties (linuron, pirimicarb, tebuconazole, iprodione,..), mostly detected in tomatoes, cucumbers and carrots.

Conclusion: Results obtained from endocrine-disrupting pesticide residue testing carried out under official controls of food are provided for an annual update of the Programme.

CONSTRUCTION AND CHARACTERIZATION OF STABLY GENE REPORTER CELL LINES FOR DETECTION OF ENDOCRINE DISRUPTORS

Dvorak Z.¹, Pavek P.², Novotna A.¹

¹Department of Cell Biology and Genetics, Faculty of Science, Palacky University, Olomouc, ²Department of Pharmacology and Toxicology, Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Hradec Kralove

Introduction: Humans are exposed increasingly to variety of endocrine disruptors from nutrition, drugs, environment etc. Most of endocrine disruptors are ligands for nuclear, steroid and xeno receptors. This is of topical interest to have reliable and high throughput tool for detection of endocrine disruptors.

Aim of the study: Our goal was to construct and characterize a panel of stably transfected gene reporter cell lines allowing detection of endocrine disruptors.

Methodology: Human hepatoma cells HepG2 and human cervix carcinoma cells HeLa were stably transfected with luciferase reporter plasmids containing responsive elements for aryl hydrocarbon receptor (AhR) and glucocorticoid receptor (GR), respectively. Transfected cells were selected on antibiotic-containing media, and the most responsive clones were characterized in detail.

Results: Detailed characterization of cell lines AZ-AHR and AZ-GR involved: (i) dose-response and time-course analyses with typical agonists, i.e. dioxin and dexamethasone, respectively; (ii) dose-response analyses with panels of ligands for AhR and GR, and with ligands for ER, AR, PR and MR to evaluate selectivity; (iii) stability of reporter cell lines over 15 passages and 30 days in culture; (iv) recovery of reporter cell lines functions after cryopreservation.

Conclusions: We have developed novel human luciferase reporter cell lines AZ-AHR and AZ-GR for monitoring AhR and GR transcriptional activities, respectively. The assays are highly sensitive, allowing high throughput format (96-well plate), and selective with potential implications for pre-clinical research and environmental applications.

Our laboratories are supported by the student project PrF 2012-002 of the Palacky University Olomouc and by the grants from the Czech Scientific Agency GACR 303/12/G163, GACR 503/10/0579 and GACR 304/10/0149.

**CURRENT SITUATION OF RESEARCH OF ENDOCRINE DISRUPTORS OCCURRING IN ARTICLES
FOR COMMON USE AND HEALTH RISKS OF CONSUMERS EVALUATION IN THE CZECH
REPUBLIC**

Sosnovcova J., Rucki M., Vrbik K.

National Institute of Public Health, Prague

Introduction: Food contact materials, toys and in articles for children can transfer their ingredients into foodstuffs or saliva and increase the human body burden from toxic chemicals. Identification of health risks demands to assemble reliable chemical analytical methods, which allow identification and quantification of selected xenobiotics with high toxicological concern.

The aim of study: The project aims to predict some important potential disruptors of the oestrogen receptor, which are contained in these product categories, using QSAR techniques. The scenario for assessment of the exposition of common population, particularly of the most high-risk group of the inhabitants (i.e. small children, immunity-deficient persons or persons with disrupted endocrine system), to the endocrine disruptors will be constructed based on the observed migration values of the compounds during real conditions of use.

Methodology: Analytical methods for determination of chemical compounds in the materials and solutions with known or predictive activity to the reproductive and hormonal system will be based on the high performance liquid chromatography with triple quadrupole detector and TOF detector.

Results: The results acquired during the investigation will provide information on contemporary burden on human organism from chemical compounds with potential toxic effects to the endocrine system by oral exposure route. There will be realistic scenario for assessment of the exposure of particular population groups to chemical compounds constructed, with focus on the most high-risk subpopulation of inhabitant.

Conclusion: Identification and quantification of xenobiotics occurring in packaging materials as a potential source of contamination of food, an estimation of exposure to these substances and subsequent measures to reduce the burden on the human body will contribute significantly to increase the level of consumer health protection.

IN VITRO EFFECTS OF SELECTED ISOFLAVONES ON TARGET GENES EXPRESSION IN HUMAN MCF-7 CELL LINE

Bialesova L., Brtko J., Macejova D.

Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava

Introduction: Breast cancer is the most common cancer in women worldwide. For over 100 years are hormones linked with development of breast cancer. Estrogen, as a main female sex hormone regulates growth and differentiation of mammary gland, ovary and uterus maturation as well as bone homeostasis. On the other hand, epidemiological studies suggested that estrogen and its metabolites may cause breast cancer development. In several plants occur chemical compounds with similar structure to estradiol molecule. They have a weak estrogenic activity and they play a role in the mechanism of action as phytoestrogens. The most studied compounds of natural origin are isoflavones and lignans. Genistein is known to exert a weak estrogenic effect by binding to estrogen receptors. Dietary intake of genistein can relieve several symptoms in postmenopausal women. Also, it has other positive effects on cardiovascular system, cancer, hyperlipidemia, osteoporosis and various forms on chronic renal disease.

The aim of study: The aim of this study was to investigate the effect of genistein alone and in combination with vitamin D or with the analogue of vitamin D, seocalcitol on expression selected nuclear receptors in human breast cancer MCF-7 cells line.

Methodology: The MCF-7 cell line was grown and passaged routinely as monolayer cultures. For experiments the cells were used at passage 10-30. Cells were seeded in Petri dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, antibiotics (penicillin, streptomycin, gentamicin) and cultured in humidified atmosphere of 5% CO₂ and 95% air at 37°C. The cells were treated 48h with the 1 µmol/l genistein and 0,1 µmol/l vitamin D and 0,1 µmol/l seocalcitol. Total RNA was isolated using Trizol reagent. Expression of selected nuclear receptors was analyzed by semiquantitative RT-PCR.

Results: Genistein, vitamin D or seocalcitol when applied alone did not affect estrogen receptor-alpha (ERalpha) expression. However, genistein in combination with vitamin D or seocalcitol caused significant diminution of ER-alpha expression. Similar effect (except Seocalcitol alone) has been found for the thyroid hormone receptor-alpha subtype.

Conclusion: Our data clearly demonstrate how a combination of cognate ligands for vitamin D receptor with isoflavone may affect expression of other important nuclear receptors.

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FOLIC ACID AND ITS IMPACT ON NEWBORNS

Zikavska T., Brucknerova I.

1st Department of Paediatrics, Faculty of Medicine, Comenius University in Bratislava

Introduction: Folates are an integral part of important biochemical processes in the human body. In the period of rapid growth of organism or cell growth, body's demands for folate are increasing. Fortification of foods with folic acid in the world is still discussed. Its impact on newborns should be variable.

The aim of the study: The presented work elaborates on the complex issue of folic acid in neonatal period. The aim of this study is to present the case of premature newborn with typical complications of prematurity and atypical laboratory findings of folate concentrations in erythrocytes. For comparison the work presents normal values of folate concentrations in erythrocytes in our group of newborns.

Methodology: The work describes the case report a premature newborn and his complications after birth. Measurement of folate concentrations in erythrocytes in umbilical cord blood with radio-immunochemical analysis shows the actual state and its reserves in organism.

Results: Patient (♂) was born in 27th gestational week (birth weight - 900 grams, birth length - 29 centimeters) with signs of asphyxia (Apgar score (5/8)). Complications of prematurity such as infections and anemia with needs of transfusions of erythrocytes were documented. Folic acid, as part of a comprehensive treatment, and also its presence in food were presented. The level of folate concentrations in erythrocytes on 1st day of life was 1214.04 ng/ml and in 11th week of life it was 5516.67 ng/ml.

Conclusion: Substitution of folic acid is one of the important parts in the comprehensive treatment of anemia in premature newborns. It is also a component of artificial milk formulas or breast milk after mother's intake. Benefits of folic acid are undoubted. On the other side excessive intake, immaturity and complications of prematurity can disrupt balance of its metabolism and it could be a potential risk for optimal development.

LAPATINIB INHIBITS MEIOTIC MATURATION OF OOCYTE – CUMULUS COMPLEXES CULTURED *IN VITRO* IN GONADOTROPINS – SUPPLEMENTED MEDIUM

Nagyova E.

Institute of Animal Physiology and Genetics Academy of Sciences of the Czech Republic, Libečov

Introduction: Epidermal growth factor receptor (EGFR) mediates the ovulatory response to LH in the ovarian follicle. The sustained activity of EGFR is a requisite for LH-induced oocyte maturation and cumulus expansion. However, abnormally elevated EGFR kinase activity can lead to various pathological states, including cancer. Several reversible and irreversible small molecule tyrosine kinase inhibitors have been developed (erlotinib, gefitinib, lapatinib, afatinib). Our work focuses on lapatinib (GW572016, Tykerb/Tyverb; GlaxoSmithKline) that reversibly and selectively inhibits the tyrosine kinase domain of both EGFR and HER2. This inhibition in EGFR-expressing and HER2-over expressing tumors blocks the activating signaling cascades in the MAPK and PI3K pathways resulting in growth arrest and/or apoptosis.

The aim of the study: was to investigate the effect of lapatinib on meiotic maturation of porcine oocyte-cumulus complexes (OCC) cultured *in vitro* in FSH/LH-supplemented medium and to determine whether inhibition of EGFR tyrosine kinase with lapatinib affects expression of the cumulus-related transcripts (tumor necrosis factor alpha-induced protein 6, *TNFAIP6*; prostaglandin-endoperoxide synthase 2, *PTGS2*), synthesis of hyaluronan (HA) and progesterone by cumulus cells. Methods performed included real-time RT-PCR, immunofluorescence and radioimmunoassay.

Results: Addition of lapatinib (10 μ M) to the culture medium blocked FSH/LH-induced nuclear maturation and cumulus expansion. The expression of *TNFAIP6* and *PTGS2* was up-regulated after 4 h in the presence of FSH/LH in comparison to control. In lapatinib-pretreated OCC, the expression of *TNFAIP6* and *PTGS2* was significantly decreased. In agreement with biochemical analyses of HA synthesis, HA was detected with biotinylated HA-binding proteins within FSH/LH-stimulated expanded OCC, with only faint HA-labeling in those treated with lapatinib. A faint cytoplasmic *TNFAIP6*-labeling pattern was detected in lapatinib-pretreated complexes. Progesterone production by OCC was significantly suppressed by lapatinib.

Conclusions: Lapatinib inhibits oocyte maturation, cumulus expansion, expression of cumulus-related genes and steroidogenesis in OCC cultured *in vitro* in FSH/LH-supplemented medium.

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HEAVY METALS AS ENDOCRINE DISRUPTORS IN THE MODEL SYSTEM

Lukac N., Forgacs Zs.¹, Knazicka Z., Lukacova J.

Department of Animal Physiology, Faculty of Biotechnology and Food Sciences Slovak University of Agriculture, Nitra, ¹National Institute of Chemical Safety, "Jozsef Fodor" National Center for Public Health, Budapest

Introduction: Endocrine-disrupting chemicals as heavy metals may interfere with the production or activity of hormones in the endocrine system, what can lead to reproductive problems such as reduced fertility and subsequently development of some diseases, including different types of cancer.

The aim of the study: The target of this *in vitro* study was to determine the effects of heavy metals, such as nickel (Ni^{2+}), mercury (Hg^{2+}) and cadmium (Cd^{2+}) on the production of sex steroid hormones in the human adrenocortical carcinoma (NCI-H295R) cell line, which serves as a model system for screening endocrine-disruptive chemicals.

Methodology: The NCI-H295R cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and passaged according to previously established and validated protocols. The cells were cultured in the presence nickel (3.9; 7.8; 15.6; 31.2; 62.5; 125; 250; 500 μM of NiCl_2), mercury (1.0; 5.0; 25; 50; 100 μM of HgCl_2), cadmium (1.9; 3.9; 7.8; 15.6; 31.2; 62.5 μM of CdCl_2) for 48 h and compared with the control group (medium without heavy metals). Enzyme linked immunosorbent assay (ELISA) was used for testosterone and progesterone quantification directly from the culture medium.

Results: Decreased hormone production was detected in all experimental groups ($P < 0.01$) with the addition of Ni^{2+} . The inhibitory effect of Ni^{2+} was already found at the lowest (3.9 μM) concentration. A concentration-dependent decline in the testosterone production was observed ($P < 0.01$) even at the lowest (1.0 μM) of Hg^{2+} concentration (4.85 ± 2.45 ng/mL). The progesterone release was also decreased as well, but this decline was less evident in comparison to the depression of testosterone. The sexual steroid production was also decreased after Cd^{2+} exposure. The lowest amount of progesterone was recorded in the groups with the highest doses 31.2 μM of Cd^{2+} ($P < 0.05$) and 62.5 μM of Cd^{2+} ($P < 0.01$), which released similar levels.

Conclusion: Our results point out a direct toxic action of heavy metals on the steroid-producing cells and subsequent changes in the concentration of testosterone and progesterone by adrenocortical carcinoma cells. Presented data showed that analyzed of heavy metals have detrimental effects on the steroid hormone synthesis even at very low concentrations, which presumably can affect their metabolites or inhibit enzymatic pathways.

DISORDERS OF THYROID AND MALE GONADS FUNCTION IN THE POPULATION OF EAST SLOVAKIA

Langer P.¹, Tajtakova M.², Kocan A.³, Drobna B.³, Huckova M.¹, Trnovec T.³, Klimes I.¹

¹*Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava,* ²*The First Clinic of Internal Medicine, P.J. Safarik University, Kosice,* ³*Department of Toxic Organic Pollutants, Slovak Medical University, Bratislava*

Introduction: Within previous 20 years several large scale surveys were conducted in highly polluted territory of eastern Slovakia, among them PCBRISK (5th framework Program od EC) and SPEECT (Slovak-US project supported by NIH).

The aim of this study: is to present a few fragments of data obtained on the interrelations of disruptors (PCBs, DDE and HCB) on the thyroid volume and function and also on the function of male gonads.

Methodology: Organochlorinated compounds in serum were estimated by gas chromatography plus mass spectrometry; hormones and antibodies by electrochemiluminiscent immunoassay; thyroid volume and structure has been examined by ultrasound. For statistical evaluation Pearson's correlation and chi-square test has been used.

Results: 1. Thyroid volume and function. In quintiles of increasing levels of $\Sigma 15$ PCBs (considered as a marker of simultaneously increasing level of DDE and HCB, which will be not shown) significant increase of following variables has been found: 1. Thyroid volume (ThV) by ultrasound Significant increase ($p < 0.001$) in the prevalence of higher thyroid volume (ThV) in quintiles of increasing $\Sigma 15$ PCBs level (which has been concordant also with the increase of DDE and HCB levels - not presented). Moreover, relatively high prevalence of positive level of thyroperoxidase antibodies (TPOab; 20-30 % in individual quintiles) has been also observed thus supporting the view that stimulatory effect of disruptors also participated by a considerable manner not only in the increase of ThV, but also in the impairment of thyroid glandular parenchyma (as will be shown by ultrasound image during the presentation) resulting in thyroid hypofunction. At the same time, about 10-15 % of increased TSH values across all quintiles show a considerable prevalence of clinically positive thyroid hypofunction related to PCBs.

Finally, significantly increasing prevalence of cases with high level of free T4 and total T3 together with TSH level decreased up to a hyperthyroid range (Attention: in hyperthyroidism TSH is decreasing due to a high level of free T4 and total T3) in hyperthyroid range which shows a rare number of cases which we previously defined as "high PCBs related subclinical hyperthyroidism" (Langer et al. – *Frontiers in Neuroendocrinol.* 31, 497-518, 2010).

2. Male gonads. In 834 males aged 20-78 years a significant decrease of testosterone (TEST) level with age has been found. However, most rapid TEST decrease appeared in 175 males between 20 and 30 years of age, while in 444 males aged 40-55 years any significant TEST decrease has not been observed.

From such reason this middle aged group of males has been selected to evaluate the effect of disruptors on TEST. As you see, there was no effect of PCBs TEST and the same was true also for DDE. However, the decrease of TEST with HCB in the same males was highly significant ($p < 0.001$). Moreover, in the same middle aged group we found very high

correlation ($p < 0.0001$) of HCB with body mass index BMI), while at the same the correlation of BMI with TEST has been highly negative ($p < 0.0001$).

Grant support: PCBRIK (5th framework Program od EC) and SPEECT (Slovak-US project supported by NIH).

COULD ENVIRONMENTAL POLLUTION WITH PERSISTENT ORGANIC POLLUTANTS AFFECT OUR METABOLIC HEALTH?

Ukropec J.¹, Radikova Z.², Huckova M.¹, Koska J.², Kocan A.³, Sebkova E.¹, Drobna B.³, Trnovec T.³,
Susienkova K.⁴, Gasperikova D.¹, Langer P.¹, Klimes I.¹

¹Diabetes Laboratory & ²Laboratory of Human Endocrinology, Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava, ³Department of Toxic Organic Pollutants, Slovak Medical University, Bratislava, ⁴Department of Statistics, Faculty of Economic Informatics, Economics University, Bratislava

Introduction: A heavily polluted area of Eastern Slovakia was targeted by the PCBRISK cross-sectional survey to search for possible links between environmental pollution and both prediabetes and diabetes.

Methods: Associations of serum levels of five persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), 2,2'-bis(4-chlorophenyl)-1,1-dichloroethylene (*p,p'*-DDE), 2,2'-bis(4-chlorophenyl)-1,1,1-trichloro-ethane (*p,p'*-DDT), hexachlorobenzene (HCB) and β -hexachlorocyclohexane (β -HCH), with prediabetes and diabetes were investigated in 2047 adults. Diabetes and prediabetes were diagnosed by fasting plasma glucose in all participants and by oGTT in 1220 compliant individuals.

Results: Entire population was stratified in terms of individual POPs quintiles and associations between environmental pollution, prediabetes and diabetes were investigated. Prevalence of prediabetes and diabetes increased in a dose-dependent manner, with individuals in upper quintiles of individual POPs showing striking increase in prevalence of prediabetes as shown by high odds ratio (OR) and 95% confidence interval (CI) for **PCBs** (2.74; 1.92-3.90), **DDE** (1.86; 1.17-2.95), **DDT** (2.48; 1.77-3.48), **HCB** (1.86; 1.7-2.95) and **β -HCH** (1.97; 1.28-3.04). Interestingly, unlike in case of PCBs, DDT and DDE, increased levels of HCB and β -HCH seemed not to be associated with increased prevalence of diabetes. Nevertheless, individuals in the 5th quintile of the variable expressing the cumulative effect of all five POPs (sum of orders) showed more than tripled prevalence of prediabetes and more than six times higher prevalence of diabetes when compared with the 1st referent quintile (individuals with the lowest levels of all 5 tested pollutants).

Conclusions/interpretation: Increasing serum concentrations of individual POPs considerably increased prevalence of prediabetes and diabetes in a dose-dependent manner. Interaction of industrial and agricultural pollutants in increasing the prevalence of prediabetes or diabetes is likely. This important finding generates an incentive for our society to act in limiting the negative effects associated with high levels of POPs in our environment.

ENDOCRINE – DISRUPTING ACTIVITIES OF PCBs AND OTHER FOOD CONTAMINANTS

Machala M., Vondracek J.

Veterinary Research Institute, Brno

Introduction: Polychlorinated biphenyls (PCBs) are important group of environmental and food contaminants, whose complex toxic modes of action include endocrine disruption (ED). The coplanar PCBs are potent ligands of the aryl hydrocarbon receptor (AhR), which contributes to disruption of various endocrine pathways. Estimation of the AhR-mediated activity of individual PCB congeners has allowed establishing of toxic equivalency factors (TEFs), suitable for their risk assessment. In contrast, toxic potencies of highly abundant “non-dioxin-like” (NDL) PCBs are not sufficiently characterized, and no accepted risk concept is currently available to estimate their toxicities, including those related to ED.

The aim of the study: Therefore, this study aimed to screen *in vitro* toxic potencies of a large set of ultrapure PCB congeners and to determine their relative importance within complex PCB mixtures.

Methodology: Relative effective potencies (REPs) were derived using *in vitro* bioassays, including determination of androgen and estrogen receptor-mediated activities, inhibition of estradiol sulfotransferase activity, binding to thyroid hormone transport protein, etc. REPs were then used to assess contribution of individual NDL congeners to total toxic potencies of mixtures.

Results: The suppression of androgen receptor-mediated gene expression was the most important toxic effect observed. Highly abundant PCBs 138, 153, 170 and 180, which also possess antiestrogenic and antithyroidal activities, were major contributors to overall endocrine-disrupting effects of PCBs, in accordance with the scientific opinion on PCBs published by EFSA.

Conclusion: Development of ED-related REPs of contaminants, such as NDL-PCBs, helps to identify the most important targets of their toxicity, and similar approaches are presently being successfully used for other emerging food contaminants. Given the extensive crosstalk among individual endocrine pathways, including e.g. neurodevelopmental and obesogenic effects, ED-related REP values of PCBs should be combined also with their neurotoxic and obesogenic potencies, in order to establish a TEF-like system also for NDL-PCBs.

RELATION OF CORD TO MATERNAL SERUM CONCENTRATION RATIOS OF INDIVIDUAL PCB CONGENERS TO THEIR PHYSICO-CHEMICAL PARAMETERS

Lancz K., Palkovicova L., Patayova H., Wimmerova S., Trnovec T.

Slovak Medical University, Bratislava

Introduction: Persistent organic pollutants readily traverse placental membranes and may present a particular risk during gestation, because thresholds for adverse effects are lowered during critical stages of fetal development.

The aim of the study: The aim of this study was to relate placental transfer of 15 PCB congeners to their physico-chemical parameters known to predict their behavior in biological systems and thus to understand better the mechanism of prenatal brain exposure.

Methodology: We included into the study 1200 births during the period 2002-2003 from two districts in eastern Slovakia with high contamination by PCBs. Concentrations of 15 PCB congeners (IUPAC No. 28, 52, 101, 123, 118, 114, 153, 105, 138, 167, 156, 157, 180, 170 and 189) in the umbilical cord and maternal serum were determined. Only values >LOD were taken into account.

Results: Regressions between maternal and cord lipid adjusted (ng/g lipids) PCB congeners serum concentration had slopes <1 (between 0.250 for PCB 114 to 0.998 for PCB 123) and $p < 0,001$, indicating some placental barrier function. Cord/maternal concentration ratios of PCB congeners were related to partition coefficient octanol-water, water solubility, total surface area of the molecule, solvent accessible surface area, electron affinity, melting point, molar volume and molecular electronegativity distance vector. The relationship between cord/maternal lipid adjusted serum concentration ratios of PCB congeners and log partition coefficient octanol-water is linear characterized by a regression coefficient = -0,415, R-square = 0,017 and $p < 0,001$. For wet weight data a regression coefficient = -0,102, R-square = 0,018 and $p < 0,001$ were obtained.

Conclusion: We have found that a single physical-chemical parameter, log KOW, largely explained the transplacental transfer for PCBs, with congeners having lower KOW preferentially transferred to the fetus.

**RELATIONSHIP BETWEEN ANTHROPOMETRIC, SOCIOECONOMIC AND HEALTH
CHARACTERISTICS OF MOTHER AND CORD/MATERNAL SERUM ORGANOCHLORINE
COMPOUNDS CONCENTRATION RATIO**

Patayova H., Wimmerova S., Lancz K., Palkovicova L., Drobna B., Hertz-Picciotto I., Jusko T. A.,
Trnovec T.

Slovak Medical University, Bratislava

Introduction: During fetal development, the placenta offers some protection against unwanted chemical exposures, but it is not an effective barrier against environmental pollutants.

The aim of the study: The aim of this study was to relate placental transfer of 15 PCB congeners to anthropometric, socioeconomic and health characteristics of mother and thus to understand better the mechanism of prenatal brain exposure.

Methodology: We included into the study 1200 births during the period 2002-2003 from two districts in eastern Slovakia with high contamination by PCBs. Concentrations of 15 PCB congeners (IUPAC No. 28, 52, 101, 123, 118, 114, 153, 105, 138, 167, 156, 157, 180, 170 and 189) in the umbilical cord and maternal serum were determined. Only values >LOD were taken into account. Cord/maternal concentration ratios (C/M) of PCB congeners were related to the following variables: Age, weight and height of mothers, parity, ethnicity, alcohol consume, illness during pregnancy, smoking during pregnancy, hypertension, respiratory diseases, rheumatoid arthritis and diabetes mellitus.

Results: Multiple regression analysis has shown that: Mothers not consuming alcohol during pregnancy had lower C/M compared to mothers consuming alcohol, except PCB 189. Healthy mothers had greater C/M compared to mothers who were ill during pregnancy. C/M increased with age of mothers. C/M increased with weight of mothers. C/M is greater in primiparas compared to secundiparas. C/M is greater in primiparas compared to tertiparas. C/M is greater in secundiparas compared to quatoparas. Romas have greater C/M compared to Caucasians, except PCB 138.

Conclusion: It can be concluded that the offspring of a mother consuming ethylalcohol is under a double attack. One is from the ethylalcohol itself and the other from developmental toxicity of organochlorines.

THE ENDOCRINE DISRUPTIVE EFFECTS OF COPPER ON THE STEROIDOGENESIS IN THE MODEL SYSTEM NCI – H295R

Knazicka Z., Lukacova J., Lukac N.

*Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture,
Nitra*

Introduction: Currently, there is increasing evidence that various chemicals introduced in the environment have the potential to cause damage to endocrine system, which regulates reproductive processes. Copper has various effects on reproductive endocrinology and it can also cause or contribute to hormonal disruption and to interfere with the key enzymes involved in steroid synthesis.

The aim of the study: The objective of our study was to determine the effects of copper (CuSO_4) on steroidogenesis in the NCI-H295R adrenocortical carcinoma cell line. Our experiment was aimed to evaluate the testosterone and progesterone production after exposure to copper.

Methodology: In our research we used the human adrenocortical carcinoma cell line (NCI-H295R) as a model system to examine the effects of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) on the production of sex steroid hormones *in vitro*. The cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured and passaged according to previously established and validated protocols. Quantification of steroid hormone was performed by the enzyme linked immunosorbent assay (ELISA) directly from aliquots of the medium after a 48 h exposure (3.9; 62.5; 250; 500; 1000 μM CuSO_4). The data were expressed in percentage of the (untreated) controls.

Results: A concentration-dependent depression in the testosterone production was observed even at the lowest concentration (3.9 μM) of CuSO_4 ($21.45 \pm 6.84\%$). The lowest testosterone levels ($P < 0.001$) were detected in the groups with the highest dose ($6.84 \pm 4.36\%$). The progesterone production was also decreased at the lowest concentration, but this decline was less evident in comparison with the depression of testosterone. The lowest amount of progesterone ($12.20 \pm 6.06\%$) was significantly ($P < 0.001$) recorded at 1000 μM CuSO_4 in comparison to the control group.

Conclusion: Our results show that copper has detrimental effects on the steroid hormone synthesis even at very low concentrations may have negative consequences on reproductive physiology. The testosterone production seemed to be more vulnerable when compared to the progesterone to copper exposure suggesting multiple sites of action for this metal in steroidogenesis.

Cu²⁺ WITH GLUCOSE IMPAIR INSULIN BINDING TO ITS RECEPTOR IN RAT LIVER PLASMA MEMBRANE

Zorad S.¹, Krskova K.¹, Gajdosechova L.¹, Dovinova I.²

¹Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava, ²Institute of Normal and Pathological Physiology Slovak Academy of Sciences, Bratislava

Introduction: Insulin, a peptide hormone, plays central role in regulation of glucose metabolism. Impaired sensitivity of target tissues to insulin – insulin resistance is a cause of several civilization diseases such as obesity, diabetes and hyperlipidemia. There is a long time search for mechanisms of insulin resistance development on insulin receptor as well as post-receptor level. Some facts, however, indicate that insulin resistance might be originated from events taking place prior insulin binding to its receptor e.g. incorrect interaction of insulin with plasma membrane outside of the receptor. Beside this the role of transient metals in “prior receptor” mechanism of insulin resistance (1) is also considered. Glucose in presence of Cu²⁺ possesses a strong oxidizing action by generating Cu⁺ which subsequently produces hydroxyl and alkoxy radicals in Fenton reaction (2). The effect of glucose on insulin binding to liver plasma membranes in presence and absence of Cu²⁺ was evaluated in present study.

Methods: Liver plasma membrane fraction was prepared by differential centrifugation. Insulin binding was performed at room temperature for 2 hrs using mono-Tyr-A14 I¹²⁵-insulin as described in (3). Glucose was used in 10 – 160 mM concentration, Cu²⁺ in 2 mM and phenanthroline, a Cu²⁺ chelator, in 2 mM concentrations.

Results: We found a linear association between glucose concentration and a decrease of specific insulin binding even in absence of Cu²⁺. This decrease was blocked by use of phenanthroline. We assume that bovine serum albumin, a high capacity metal chelator used in insulin binding assay, is the source of Cu²⁺. Addition of exogenous Cu²⁺ restored the insulin binding inhibiting action of glucose in presence of phenanthroline. Pre-incubation of insulin with glucose in presence of Cu²⁺ was without effect on subsequent hormone binding.

Conclusion: Glucose in presence of transition metals especially Cu²⁺ generates reactive oxygen species (ROS). ROS might attack cell plasma membrane lipids and proteins including insulin receptor. Impaired insulin receptor function caused by Cu²⁺ and glucose *in vitro* emphasizes the possible role of transition metals in pathogenesis of diabetes.

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EFFECT OF COPPER COMPLEX ON DNA CLEAVAGE AND TOXICITY *IN VITRO*

Dovinova I.¹, Gajdosechova L.², Zorad S.²

¹*Institute of Normal and Pathological Physiology Slovak Academy of Sciences, Bratislava*, ²*Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava*

Introduction: Transition metals like iron and copper are characterised by their ability to change valence, coordinates redox status of metal proteins and/or promote free radical production. The copper is 7 - times more reactive than iron, however its level is 50 - times lower. In physiological conditions copper is tightly bound in chelate centers of protein, such as ceruplasmin, several enzymes of electron transport chain, on albumin and others. Copper underlies reversible reduction according its binding strength and participates in several redox reactions. In our study we focused on effect of copper complex on DNA cleavage and influence on leukemic cells surviving.

Methods: **DNA cleavage** was determined after incubation of DNA plasmid with tested substances and detected bands between superspiralised and relaxed plasmid DNA was separated in 2% agarose gel, visualized using ethidium bromide and UV detection. **Cytotoxicity** was determined using MTT chemosensitivity test and cell surviving was detected after staining cells with trypan blue. **Apoptosis** of leukemic cells were determined by DNA ladder electrophoresis or using FACs analysis after FDA/PI staining. **Membrane integrity damage** have been observed after PI staining by FACs analysis.

Results: Effect of copper complex on DNA cleavage was highly increased in the presence of hydrogen peroxide and GSH. Cu-complex potentiates one strand cleavage of DNA 25% times. Cytotoxicity of copper complex was lower than 0.1uM, however no changes in cell cycle have been observed. Main observed mechanism was membrane integrity damage and subsequent apoptosis induction. In combine therapy with antimetabolite AraC as a main chemotherapeutics, copper complex potentiated decrease of leukemic cell amount either by additive or by synergic manner.

Conclusion: Copper complex can act as toxic and anticancer substance by DNA cleavage, however their main mechanism *in vitro* is performed through membrane disintegration and induction of apoptosis.

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PRENATAL A POSTNATAL PCB EXPOSURE OF NON – BREASTFED INFANTS FROM MICHALOVCE DISTRICT AND THEIR NEURODEVELOPMENT

Fabisikova A., Drobna B., Conka K., Chovancova J., Dömötöröva M., Kocan A.

Department of Toxic Organic Pollutants, Faculty of Medicine, Slovak Medical University, Bratislava

Introduction: Polychlorinated biphenyls (PCBs) are ubiquitous, persistent, bio-accumulative environmental pollutants. Exposure of children to these pollutants can occur prenatally through passage across the placenta, or postnatally by lactation or food consumption. In infant period, the major food sources are breast milk and infant milk formula.

Objective: The aim of this study was to examine pre- and postnatal PCB exposures of non-breastfed infants and children's mental and psychomotor development at 10 months of age.

Methods: Fifty-five non-dioxin-like and dioxin-like mono-ortho substituted PCB congeners were determined in six brands of infant milk formula declared by 9 mothers used for their infants and in all obtained maternal (n=9), infant's (n=9) and cord blood (n=8) serum samples by ¹³C₁₂-isotope-dilution method using high resolution gas chromatography – high resolution mass spectrometry (HRGC/HRMS MAT 95 XP). The mental and psychomotor development of each infant was assessed at 10 months using the Bayley Scales of Infant Development.

Results: The levels of total PCBs in 6 formula milk samples ranged from 0.63 to 1.97 ng/g lipid, with a mean value of 1.24 ng/g lipid. The mean (range) concentration of total PCB in maternal, cord blood and infant's serum was 958 (178-3245) ng/g lipid, 979 (128-3212) ng/g lipid and 232 (34-1270) ng/g lipid, respectively. Child with the highest level of PCBs with mild delay in mental development belongs to mother with the highest level of PCBs. However no adverse effects of PCBs on psychomotor neurodevelopment of infants at the age of 10 month were observed in this study.

Conclusion: Formula milk lipids contain a negligible amount of PCBs and the postnatal exposure of PCBs in formula-fed infants is no cause for concern although they were prenatally exposed to some extent since PCBs can pass through the placental barrier. Mothers residing in Michalovce are still highly exposed to PCBs, and their body burdens of these pollutants may pose a risk for adverse effects on health for themselves and their children.

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PERSISTENT ORGANIC POLLUTANTS IN FOOD SAMPLES FROM POTENTIAL SOURCES OF CONTAMINATION IN SLOVAKIA

Conka K., Fabisikova A., Chovancova J., Dömötöröva M., Stachova Z., Drobna B., Kocan A.

Slovak Medical University, Bratislava

Introduction: Persistent organic pollutants (POPs) such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are toxic compounds with various adverse effects on human health.

The aim of the study: Within our project we focused on sites with chemical, metallurgical plants and waste incinerators in Slovakia that are potential sources of contamination of environment with POPs (Košice, Krompachy, Nemecká, Šaľa). In these four areas and one background area (Starina) in total 163 food samples in 2006 - 2007 were collected.

Methodology: Various extraction methods depending on sample type were used for fat extraction. Analyses of seventeen 2,3,7,8-substituted PCDDs/PCDFs, four non-ortho and eight mono-ortho PCBs were performed in accordance with the USEPA 1613 and 1668 isotope dilution methods using high resolution gas chromatography coupled with high resolution mass spectrometry in the selected ion monitoring mode.

Results: The minimum and maximum levels of the sum of PCDDs, PCDFs and dl-PCBs expressed as toxic equivalent (TEQ) in pg WHO₁₉₉₈TEQ g⁻¹ fat were 0.04 – 9.16 for the samples of pork origin, 0.59 – 32.6 for milk samples, 0.17 – 181 for hen egg samples and 43.5 for duck fat.

Conclusion: Our study was concentrated on the impact of selected POP sources on the environmental contamination and the transfer to feed and food. The highest levels of PCDDs and PCDFs were observed in eggs and milk samples from Krompachy region and in pigs from Starina and Nemecka region. The highest levels of PCBs were observed in eggs from Nemecka region, in pigs from Košice region and in milk from Krompachy region.

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EFFECTS OF SELECTED ENDOCRINE DISRUPTORS ON PORCINE OOCYTE-CUMULUS COMPLEX EXPANSION AND OOCYTE MATURATION

Mlynarcikova A., Fickova M., Scsukova S.

Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava

Introduction: Environmental exposure to plasticizers with endocrine disrupting activities, such as phenols and phthalates, is suspected to produce undesirable effects on health, including fertility problems. These agents can affect reproductive functions at different sites, e.g. due to their presence in the ovarian follicles, follicular cell physiology could be impaired.

The aim of the study: We analyzed effects of two phenols: bisphenol A (BPA) and 4-chloro-3-methyl phenol (CMP); and two phthalates: di(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP), on the processes important for the release of fertilizable oocyte: expansion of porcine oocyte-cumulus complex (OCC) corresponding to hyaluronan (HA) synthesis by cumulus cells, and nuclear maturation of oocytes.

Methodology: OCC were isolated from preovulatory porcine ovarian follicles. After 24 h incubation with the tested compounds (10^{-8} to 10^{-4} M), expansion of the cumulus cells induced by follicle-stimulating hormone (FSH) (1 μ g/ml) was assessed according to a subjective score system. HA synthesis by cumulus cells was measured using radiometric precursor assay. After 44 h culture, nuclear maturation of porcine oocytes was assessed after orcein staining.

Results: The highest concentration of BPA inhibited cumulus expansion as a result of disrupted HA retention within the matrix of the expanding OCC. Similar inhibition of cumulus expansion was observed also for CMP and BBP. BPA and CMP also inhibited meiotic maturation of the oocytes suppressing them to reach metaphase II stage of meiosis.

Conclusion: From the present study it could be assumed that studied ovarian intrafollicular processes might be considered as possible endpoints by which endocrine disruptors could exert their negative effects on female fertility.

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EFFECTS OF SELECTED ENDOCRINE DISRUPTORS ON STEROIDOGENESIS IN OVARIAN CELLS

Scsukova S., Fickova M., Mlynarcikova A.

Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava

Introduction: The research in the field of reproductive toxicity confirms that gonadal processes are sensitive to the action of exogenous compounds from environment, cosmetic and pharmaceutical products. Despite the structural diversity of exogenous natural and synthetic estrogens, all of them have the capacity to bind to estrogen receptors in target cells of the body and initiate or inhibit estrogen-like actions.

The aim of the study: This study was designed to compare the *in vitro* effects of natural estrogens and selected phenol and phthalate derivatives on steroid hormone production by ovarian granulosa cells.

Methodology: Granulosa cells isolated from porcine ovarian follicles (4-6 mm) were incubated with the tested compounds (10^{-13} – 10^{-4} M) in the presence or absence of follicle-stimulating hormone (FSH) (1 µg/ml) for different time periods (24, 48, 72 h). At the end of the incubation, progesterone and estradiol levels released to the culture media were measured by radioimmunoassay.

Results: Natural estrogens and most of the tested phenols exerted a significant inhibitory effect on basal as well as FSH-induced progesterone synthesis by granulosa cells at 10^{-5} or 10^{-4} M concentrations. The most effective phenol was bisphenol A. The reduced progesterone levels were not induced by the cytotoxic effect of the tested compounds as revealed by LDH assay. Estradiol synthesis in granulosa cells was not affected by the action of the tested phenols. On the contrary, di(2-ethylhexyl) phthalate (DEHP) stimulated basal progesterone and estradiol production by granulosa cells in a concentration-dependent manner.

Conclusion: We suppose that alterations in steroid hormone production by ovarian granulosa cells induced by particular endocrine disruptors may not necessarily be mediated through competitive binding with estrogen receptor but the enzymes involved in steroidogenesis might be implicated in the action of these agents.

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IMPACT OF POLYMERIC NANOPARTICLE PEG-*b*-PLA ON RAT HYPOTHALAMIC-PITUITARY-GONADAL FUNCTIONS

Rollerova E.¹, Scsukova S.², Jurcovicova J.², Mlynarcikova A.², Kiss A.², Wsolova L.¹, Kovriznych J.¹, Zeljenkova D.¹, Szabova E.¹, Vavra I.³, Ciampor F.⁴, Sadlonova I.⁵

¹Department of Toxicology, Slovak Medical University, Bratislava, ²Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava, ³Institute of Electrical Engineering Slovak Academy of Sciences, Bratislava, ⁴Institute of Virology Academy of Sciences, Bratislava, ⁵Hameln rds a.s., Modra

Introduction: Within the context of nanomedicine, the polymeric nanoparticles poly(ethylene glycol)-*block*-poly(lactic acid) (PEG-*b*-PLA) were developed for delivery poorly water-soluble CNS drugs that have potential to cross blood brain barrier and exert direct action upon the brain.

The aim of the study: Our recent results have shown that neonatal exposure to PEG-*b*-PLA significantly accelerated the onset of puberty, induced estrous cycle irregularity in 54% of female rats, affected progesterone levels and significantly increased pituitary weight. The purpose of present study was to evaluate histological pattern of pituitary and to examine immunolocalization of pituitary prolactin synthesis in adult female rats neonatally exposed to PEG-*b*-PLA.

Methodology: Neonatal female Wistar rats were intraperitoneally injected with 20 and 40 mg/kg b.w. of PEG-*b*-PLA, 4 µg/kg b.w. of diethylstilbestrol (DES, positive control) or vehicle alone (ultrapurified water) from postnatal day 4 to 7. Animals were euthanized with ketamin/xylasine and sacrificed by decapitation on the closest age after postnatal day 176 at which estrus occurred. Pituitaries following formalin fixation and haematoxylin/eosin staining were subjected to histopathological examination. Localization of prolactin in pituitary cells was quantified using single immunohistochemistry.

Results: Microscopic structure of pituitary corresponded to physiological morphology of the organ. The difference between both PEG-*b*-PLA-treated groups and both control groups in the level of hyperaemia has been observed. There was obvious middle hyperaemia of blood vessels and capillaries in low dose PEG-*b*-PLA group (20 mg/kg b.w.) and slight hyperaemia in high dose PEG-*b*-PLA group (40 mg/kg b.w.). Preparations in control exerted only sporadic hyperaemia similarly to DES group where no hyperaemia was observed. There was no difference in prolactin cytoplasmic localization among all compared studied groups.

Conclusion: The results indicate that pituitary hypertrophy might coincide with hyperaemia as an effect of nanoparticle tested.

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EFFECTS OF VINCLOZOLIN ON SELECTED NUCLEAR RECEPTORS AND THEIR COREGULATORS

EXPRESSION IN HUMAN MCF-7 CELLS

Macejova D., Fickova M., Brtko J.

Institute of Experimental Endocrinology Slovak Academy of Sciences, Bratislava

Introduction: Endocrine disruption refers to the interference of endocrine system function by environmental chemicals. Compounds, such as Vinclozolin, belong to a group of chemicals known as endocrine disruptors. Vinclozolin is a non-systemic fungicide of the dicarboximide group, registered for both pre- and post-harvest use on fruits, vegetables and ornamental plants to control *Botrytis spp.*, *Sclerotinia spp.*, *Monilia fruticola* and *Gloeosporium spp.*

Retinoids, rexinoids, vitamin D, as well as thyroid hormone play important role in regulation of growth, differentiation, metabolism and morphogenesis in higher vertebrates and humans. These effects are mediated through their nuclear retinoic acid receptors (RARs, RXRs, TRs, VDR) and their coregulators. It is well known that nuclear receptors of hormones and biologically active ligands can be affected by a number of endocrine disrupting chemicals.

The aim of the study and methodology: The aim of present study was to investigate the *in vitro* effects of Vinclozolin on expression of nuclear RAR and RXR (RARalpha, beta, gamma and RXRalpha, beta), TR (TRalpha, beta) subtypes and their coregulators (SMRT and SRC-1) in MCF-7 cell line treated with the above compound at the concentration of 1 µmol/l for 24 and 72 h. The expression of selected nuclear receptors and their coregulators has been analyzed by the semiquantitative RT-PCR technique.

Results: Our data has shown that Vinclozolin enhanced expression of RARalpha, RARbeta and RARgamma in MCF-7 cells after 24 h treatment. On the other hand, 72 h treatment with Vinclozolin resulted in enhanced expression of RARgamma and RXRbeta and reduced expression of RXRalpha. TRalpha expression has been enhanced in MCF-7 cells either after 24 h or 72 h treatment. Vinclozolin also exerted enhanced TRbeta expression after 72 h treatment when compared to untreated cells. Moreover, Vinclozolin was found to enhance expression of both coregulators of nuclear receptors SMRT and SRC-1 in MCF-7 cells after 24 h treatment. Expression of VDR was unaffected by this compound.

Conclusion: These results from *in vitro* experiments suggest that these compounds may play a marked role in modulation of expression of retinoid, rexinoid and thyroid hormone receptors as well as their coregulators in MCF-7 cells.

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THE EFFECT OF 4-NONYLPHENOL, AN ENDOCRINE DISRUPTOR, WITH THE PROTECTIVE EFFECT OF 17 β -ESTRADIOL ON SPERMATOZOA *IN VITRO*

Lukacova J., Knazicka Z., Tvrda E., Lukac N.

Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Nitra

Introduction: Nonylphenol (NP) is a toxic compound capable of interfering with the hormonal system of numerous organisms. Estrogens play a central role in female reproduction, but also affect the male reproductive system. In males, it is present at extraordinarily high levels in semen. Estrogens stimulate mammalian sperm capacitation, acrosome reaction and fertilizing ability.

The aim of the study: The aim of this study was to evaluate the negative effect of NP and the protective effect of 17 β -estradiol against the influence of NP on bovine spermatozoa motility during several time periods.

Methodology: The bovine spermatozoa were incubated with various concentrations of NP dissolved in 1% dimethyl sulfoxide (DMSO; concentrations 1; 10; 100; 200 $\mu\text{g}/\text{mL}$ of NP) with or without addition 1 $\mu\text{g}/\text{mL}$ 17 β -estradiol dissolved in 1% ethanol. The control group (medium without NP) was compared to the experimental groups (exposed to different concentrations of NP) and to the experimental groups (exposed to different concentrations of NP with addition 17 β -estradiol) during 0 h, 2 h, 4 h and 6 h of *in vitro* cultivation. The spermatozoa motility was determined by CASA (Computer Assisted Semen Analyzer) system using the Sperm VisionTM program.

Results: The average values of the spermatozoa motility were lower in all experimental groups with the addition of NP during all time periods. Significant differences ($P < 0.001$ and $P < 0.05$) between the control group and all experimental groups were recorded. The lowest motility of spermatozoa was found at the doses $> 100 \mu\text{g}/\text{mL}$ of NP (67.36%; 62.39%) in comparison to the control group (77.41%) after 6 h of *in vitro* cultivation. The addition of 17 β -estradiol increased the spermatozoa motility, but the results were not significant ($P > 0.05$).

Conclusion: Currently, nonylphenol markedly negatively affects male reproductive system. Our results prove the pronounced impact of higher concentrations of NP on the spermatozoa motility and the protective effect of 17 β -estradiol against the action of NP.

PAN-EUROPEAN CAMPAIGNS ON EDCs IN GROUND-, SURFACE- AND WASTE-WATERS

Blaha L., Jarosova B., Benisek M., Hilscherova K., Giesy J., Loos R., Gawlik B.

RECETOX, Masaryk University, Brno

Introduction: Numerous individual chemicals have been identified as endocrine disrupting compounds (EDCs) affecting both human health and natural biota. There are, however, many other compounds present in complex environmental mixtures, including also transformation products, which have poorly been characterized. Therefore, there is a need to assess effects of the whole environmental matrices (e.g. waters) at national- and international levels, to fully characterize potential risks of EDCs.

The aim of the study: To characterize levels of emerging aquatic contaminants (including EDCs) within Pan-European snap-shot monitoring campaigns by combining targeted chemical analyses and whole-extract biological assessment for ED-activities.

Methodology: Several sampling campaigns have been coordinated by EC JRC Ispra. The campaigns (in total 337 samples from 30 EU countries) covered groundwaters, surface waters as well as effluents and wastewater sludge. Samples have been collected within specified time windows to provide a "snapshot" information about the EU-wide situation, and distributed to several cooperating laboratories that provided chemical analyses of traditional and emerging pollutants (more than 200 analytes) and biological assessment for estrogenicity and dioxin-like toxicity (*in vitro* reporter gene assays MVLN and H4IIE.luc).

Results: The presentation provides an overview of the campaigns and their results. For the sake of clarity only example results are given here on waste waters that have been confirmed as highly relevant source of EDCs and other emerging aquatic contaminants. The most relevant compounds identified in the effluent water samples in terms of frequency of detection, maximum, average and medium concentration levels were artificial sweeteners (Sucralose, Acesulfame K), perfluoroalkyl substances (PFOA, PFHxA, PFHpA, PFOS), insect repellent DEET, benzotriazoles (corrosion inhibitors), pharmaceuticals (Bisoprolol, Carbamazepine, Ciprofloxacin and many others), organo-phosphate ester flame retardants (TIBP, TBP, TCEP and others), x-ray contrast media (amidotriazoic acid, Iohexol, Iopromid, etc.), pesticides (Terbutylazine, MCPA, Mecoprop, Diuron) or Triclosan (antibacterial). The biological effects included estrogenicity: from the total number of 75 WWTP effluents, 27 sample extracts showed significant estrogenic activity with maxima up to 18 ng/L estrogen equivalents (EEQ), which highly exceeds EQS limits (0.05 ng/L for ethinylestradiol) suggested by a novel EU legislation for waters. Twenty one samples also elicited dioxin-like activity but with relatively low effect levels (only up to 0.4 ng TEQ equivalents per liter).

Conclusion: The elimination of most of the anthropogenic substances, including EDC compounds such as estrogens, in conventional WWTPs with secondary biological treatment is incomplete and improvements of wastewater treatment and subsequent treatments of the produced sludge are required.

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ENDOCRINE DISRUPTIVE POTENTIAL OF CYANOBACTERIAL METABOLITES

Hilscherova K., Novakova K., Jonas A., Sychrova E., Fetter E., Scholz S.

RECETOX, Masaryk University, Brno

Introduction: The presence of endocrine disruptive compounds in water is mostly discussed solely in relation to anthropogenic pollutants. However, compounds released by cyanobacteria and their specific toxic potencies can be of high environmental relevance namely in case of mass expansion of cyanobacterial water blooms which are a major problem in many water bodies around the world. The hazardous potential of cyanobacterial metabolites can be caused by both extracellular products directly entering the environment, but also by substances present inside the cyanobacterial cells. Intracellular compounds may impact on organisms via the food chain or after their release during the degradation of cyanobacteria.

The aim of the study: The primary goal of our studies was to determine if cyanobacterial species can produce compounds which could interfere with endocrine system. Our studies evaluate the endocrine disruptive (ED) potential of metabolites produced by cyanobacteria and algae. The research focused on both the exudates produced during the growth phase of cyanobacteria and the extracts of the cyanobacterial cells.

Methodology: Cytotoxicity and specific toxic potencies of products of freshwater cyanobacteria were determined by use of *in vitro* reporter gene trans-activation assays. In the first study, several ED modes of action were investigated for samples from field and laboratory cyanobacteria biomasses. In further research, estrogenic potency of aqueous extracts and exudates (culture spent media with extracellular products) of several species of cyanobacteria and algae was assessed in detail by use of *in vitro* assays. Finally, a transgenic zebrafish strain (cyp19a1b-GFP, Brion et al., PlosOne 2012, e36069) was used to assess the estrogenicity of products from selected species *in vivo*. This strain expresses green fluorescent protein under the control of the aromatase B (cyp19a1b) promoter, which is induced by estrogens.

Results: Estrogenic potency was detected in most cyanobacterial environmental samples. Some of them also showed potency to interfere with the signaling of androgen receptor. In laboratory studies, compounds produced by cyanobacteria and algae, and in particular those excreted from the cells, were estrogenic. The estrogenic potency was not correlated with concentrations of known cyanotoxins, such as microcystin and cylindrospermopsin, which suggests that the estrogenic potency was caused by other compounds. The extracts and exudates from tested algae species also exhibited significant estrogenicity, which indicates the role of compounds not specific for cyanobacteria, in the observed effects. *In vivo* assay with fish embryos documented an increase in estrogenic response compared to control in embryos exposed to 3 and more times concentrated exudates of selected cyanobacteria and algae. These concentrations also caused malformations including deformities of the tail, mouth or edemas.

Conclusion: The demonstrated estrogenic potency of compounds present in cyanobacterial biomasses is of environmental relevance, and could potentially contribute to endocrine disruptive effects in aquatic ecosystems, namely in case of greater bloom densities.

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